REMARKS

Claims 1-3, 10-14, 20, 22-26, and 30-51 are currently pending in the application. Claims 1, 14, 36, 40, 44, and 50 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

At the outset, Applicants would like to thank Examiner Hutson for taking the time to discuss the contents of this Office Action with Applicant's representative on January 30, 2006. The suggestions made by Examiner Hutson have been incorporated into this response.

Claim Objections

The Office Action states that claims 2 and 13 are objected to because they depend from rejected claims. Because prosecution is ongoing, Applicants are not taking any action at this time with respect to claims 2 and 13, but in the event that no allowable subject matter is found in the claims from which claims 2 and 13 depend, Applicants will take appropriate action to either cancel or amend claims 2 and 13.

Rejection of Claims 1, 3, 10-12, 14, 20, 22, 44, and 50 Under 35 U.S.C. §112, Second Paragraph

The Office Action states that claims 1, 3, 10-12, 14, 20, 22, 44, and 50 are rejected under §112, second paragraph for alleged indefiniteness. The Office Action states that claims 3, 14, 44, and 50 are indefinite because of the insertion of "SEQ ID NO: 10" into the claim. The Office Action states that the relationship of SEQ ID NO: 10 to JDF-3 DNA polymerase is unclear.

The claims were amended in Applicants' response of December 1, 2004 to include reference to the JDF-3 DNA polymerase amino acid sequence as suggested by the Examiner during a telephone interview between the Examiner and Applicants' representatives on June 21, 2004. Page 18 of the specification was amended to incorporate the amino acid sequence of JDF-3 from PCT application publication number WO 01/32887 into the specification, and the sequence was designated by the sequence identifier: SEQ ID NO: 10. Accordingly, it is clear from the specification as amended that SEQ ID NO: 10 is the amino acid sequence of JDF-3

DNA polymerase and, thus, the relationship between JDF-3 and SEQ ID NO: 10 is clear and unambiguous. To further clarify the relationship between JDF-3 and the sequence of SEQ ID NO: 10, claims 3, 14, 44, and 50 have been amended to recite "JDF-3 DNA polymerase having the sequence shown in SEQ ID NO: 10."

The Office Action states that claims 1, 10-12, 20 and 22 are rejected as allegedly indefinite in their recitation of an enzyme that comprises DNA polymerization activity. Claims 1 and 12 have been amended to clarify that the first enzyme has the DNA polymerization activity of a DNA polymerase or a reverse transcriptase. Applicants believe that this amendment should obviate the rejection.

Applicants request that the rejections be reconsidered and withdrawn.

Rejection of Claims 1, 10-12, 20, 22, 36-39, 40-43, 44-47, and 48-51 Under 35 U.S.C. §112, First Paragraph

The Office Action states that claims 1, 10-12, 20, 22, 36-39, 40-43, 44-47, and 48-51 under §112, first paragraph for allegedly failing to comply with the written description requirement. The Office Action states that claims 1, 10-12, 20, and 22 are directed "to all possible enzyme mixtures comprising a first enzyme and a second enzyme wherein said first enzyme is any enzyme which comprises any DNA polymerization activity." The Office Action states that the specification fails "to sufficiently describe the claimed invention, in such full, clear, concise, and exact terms that a skilled artisan would recognize Applicants were in possession of the claimed invention." Applicants respectfully disagree and traverse the rejection.

The claims recite an enzyme that comprises "a DNA polymerization activity." In support of this recitation, the specification teaches over 30 examples of specific enzymes that fall under this limitation. The specification teaches 16 different DNA polymerases that fall under the claim, 9 different reverse transcriptases, and teaches further numerous examples of mutations that can be made to enzymes that permit the enzyme to retain its DNA polymerization activity. If the Examiner does not agree that this is a representative number of species of enzyme having DNA polymerization activity to support the claim, Applicants request that the Examiner indicate

how many examples of enzymes having DNA polymerization activity would be a representative number. The Office Action suggests that because the enzymes disclosed in the specification can be grouped into two subgenera, and that because the specification does not describe other subgenera of enzymes having DNA polymerization activity, that the claims do not meet the written description requirement. The written description requirement "may be satisfied through sufficient description of a representative number of species...[s]atisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession ...of the genus." The question for determining whether the claims meet the written description requirement is, in part, whether the specification teaches enough species to evidence that Applicants, in view of the state of the art, were in possession of the invention, not whether the specification describes a sufficient number of sub-genera.

One of skill in the art would have no reason to doubt that Applicants were in possession of an enzyme comprising "a DNA polymerization activity." As described in further detail below, enzymes that have DNA polymerization activity have been known in the art for almost 20 years. A search of PubMed using the term "DNA polymerization" returned over 2000 references, dated as early as 1960. Moreover, the specification provides over 30 specific examples of enzymes that comprise a DNA polymerization activity. Thus, one of skill in the art, reading the phrase "said enzyme comprises a DNA polymerization activity" would have a clear view of exactly what enzymes would fall under this claim. Nevertheless, solely for the purpose of advancing prosecution, claim 1 has been amended to recite "said first enzyme comprises a DNA polymerization activity of a DNA polymerase or reverse transcriptase." This amendment should obviate the rejection. As amended, the instant claims satisfy the written description requirement, and Applicants accordingly request that the rejection be reconsidered and withdrawn.

The Office Action states that claims 36-39, 40-43, 44-47, and 48-51 are rejected for lack of written description "because each of these claims recite that the first enzyme is a Taq DNA polymerase...a KOD DNA polymerase...or a "JDF-3 DNA polymerase...when applicants have only described those Taq, KOD, and JDF-3 DNA polymerases described on page eleven of applicants specification." The Office Action states that "[a]s an organism contains more than

one type of DNA polymerase, applicants have not described those DNA polymerases in addition to the single species on page eleven." Applicants respectfully traverse the rejection.

With the respect to the recitation of a JDF-3 DNA polymerase in claims 44 and 50, these claims were amended previously to indicate that the claim refers to the JDF-3 sequence of SEQ ID NO: 10. The claims have been amended again herein, to make clear that the claims refer to JDF-3 DNA polymerase "having the sequence of SEQ ID NO: 10." Accordingly, Applicants believe that the rejection is moot with respect to claims 44, 50, and their dependents.

Claim 36 has been amended to recite "Taq DNA polymerase" instead of "a Taq DNA polymerase" at the suggestion of Examiner Hutson, to clarify that the claim refers to the Taq DNA polymerase that is well known to those of skill in the art, and commercially available from numerous vendors. For example, there are over 30 issued U.S. patents by David Gelfand and Cetus corporation relating to the discovery and use of Taq polymerase , the earliest of which was issued in 1989. There are over 2400 literature references that refer to Taq polymerase, and date back to well before the filing date of the instant application. In addition, there are at least 20 commercial vendors of Taq polymerase (as shown in Exhibit A) from which one of skill in the art could obtain Taq for making the claimed invention. Accordingly, at the time the instant application was filed, one of skill in the art would have understood reference to "Taq DNA polymerase" to refer to the Taq polymerase (either native or recombinant) that was known in the art at the time, and that was readily commercially available.

Claim 40 has been amended to recite "KOD polymerase" instead of "a KOD polymerase" to clarify that the claim refers to the KOD DNA polymerase that is well known to those of skill in the art, and commercially available from several vendors. For example, the discovery, characterization, and use of KOD DNA polymerase is described in seven issued U.S. patents (issued as early as 1999), and more than 20 literature references dating back to 1997. In addition, KOD DNA polymerase is readily commercially available from vendors such as Novagen (Exhibit B), Invitrogen (Exhibit C), and Toyobo Co., Ltd. (Exhibit D). Accordingly, at the time the instant application was filed, one of skill in the art would have understood reference

to "KOD DNA polymerase" to refer to the KOD polymerase that was known in the art at the time, and that was readily commercially available.

Accordingly, one of skill in the art, would have understood "Taq DNA polymerase" or "KOD DNA polymerase" as recited in the claims to refer to the Taq and KOD DNA polymerases that were known in the art at the time the instant application was filed, and that were described in both the patent and non-patent literature available as of the instant filing date. The claims, therefore, describe the invention in sufficient detail to evidence to one of skill in the art that Applicants were in possession of the claimed invention. Applicants therefore request that the rejection be reconsidered and withdrawn.

Rejection of Claims 1-3, 10-14, 20, 22, and 36-51 for Obviousness-type Double Patenting

The Office Action states that claims 1-3, 10-14, 20, 22, and 36-51 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 64-70 and 75-87 of USSN 10/079,241. Without acquiescing to the rejection, Applicants intend to overcome this rejection by providing a Terminal Disclaimer in the instant case upon the notification of allowable claims.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

Date: March 31, 2006

Name: Kathleen M. Williams
Registration No.: 34,380
Customer No.: 27495
Edwards Angell Palmer & Dodge LLP
P.O. Box 55874
Boston, MA 02205
Tel. (617) 239-0100

Exhibit A

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AmpliTaq® DNA Polymerase, LD with Buffer II Applied Biosystems request info Promotion Promotion	Concentration: 5 U/µL Size: 250 U	GET QUOTE BUY NOW
Biolase DNA Polymerase Bioline request info Promotion	Concentration: 5 U/µl Size: 500 U	$135.00 BUY NOW
Mango-Taq DNA Polymerase Bioline request info Promotion	Concentration: 1 U/µl Size: 2000U	$285.00 BUY NOW
Taq DNA Polymerase, 1000 U (4 x 250U) without dNTP Bioneer request info	Concentration: 5,000 U/ml Size: 4 x 250 U	GET QUOTE
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	request info		
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	Taq DNA Polymerase Recombinant Invitrogen request info	Concentration: 5U/µl Size: 500 U	BUY NOW
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	MoBiTaq Polymerase (K) MoBiTec GmbH request info	Concentration: 25 U/µl Size: 1000 U	GET QUOTE BUY NOW
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	Taq DNA Polymerase Molecular Cloning Laboratories (MCLAB) request info	Size: 5 x 200 U Concentration: Request Info Form: Request Info	$175.00
	Taq DNA Polymerase Molecular Cloning Laboratories (MCLAB) request info	Size: 10 x 200 U Concentration: Request Info Form: Request Info	$350.00
	Taq DNA Polymerase Thermus aquaticus Sigma-Aldrich request info	Size: Request Info Concentration: Request Info Form: Request Info	GET QUOTE BUY NOW
	Taq DNA Polymerase Thermus aquaticus Sigma-Aldrich request info	Size: Request Info Concentration: Request Info Form: Request Info	GET QUOTE BUY NOW
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Exhibit B

- Novagen Product Spotlights
- Product Spotlights
- KOD DNA Polymerases
  - KOD Citations by Application
  - **Overview**
  - KOD HiFi
  - KOD Hot Start
  - KOD XL

KOD DNA Polymerases Overview

- KOD HiFi DNA Polymerase
- KOD Hot Start DNA Polymerase
- KOD XL DNA Polyerase
- KOD Citations by Application

Back to KOD main page

KOD DNA Polymerases Overview

We offer a complete selection of Novagen® enzymes and kits for PCR, featuring KOD HiFi DNA Polymerase. This unique proofreading enzyme, isolated from the extreme thermophile *Thermococcus kodakaraensis* KOD1, possesses superior processivity and fidelity that enables faster and more accurate PCR amplification than can be achieved with conventional enzymes, including *Pfu* DNA polymerase (1). KOD HiFi DNA Polymerase is also available in a hot start version for high specificity and increased read length (2), and as a blend (KOD XL DNA Polymerase) recommended for very long templates (3).

	DNA Polymerase		
	KOD HiFi	*Pfu*	*Taq*
Species	*Thermococcus kodakaraensis*	*Pyrococcus furiosus*	*Thermus aquaticus YT-1*
Fidelity*	0.0035	0.0039	0.013
Elongation rate (bases/second)	106–138	25	61
Processivity (nucleotide bases)	>300	< 20	not determined

*Fidelity was measured by the authors as mutation frequency in PCR products using a sensitive blue/white phenotypic assay using a 5.2 kbp *lacZ* plasmid as template (1).

PCR Enzyme Selection Guide

Enzyme	PCR Product Size kbp	Elongation Rate bases/s	Specificity	Fidelity	GC-rich Templates	Yield	PCR Product Ends
KOD Hifi DNA Polymerase	< 6	120	●	■		■	blunt
KOD Hot Start DNA Polymerase	< 21	120	■	■	■	■	blunt
KOD XL DNA Polymerase	< 30	120	●	■	■	■	Mixed (blunt and 3'-dA)

● Satisfactory ▲ Good ■ Excellent

1. Takagi, M., et al. (1997) *Appl. Environ. Microbiol.* **63**, 4504–4510.
2. Mizuguchi, H., Nakatsuji, M., Fujiwara, S., Takagi, M., and Imanaka, T. (1999) *J. Biochem.* (Tokyo) **126**, 762–768.
3. Nishioka, M., Mizuguchi, H., Fujiwara, S., Komatsubara, S., Kitabayashi, M., Uemura, H., Takagi, M., and Imanaka, T. (2001) *J. Biotechnol.* **88**, 141–149.

Exhibit C

Invitrogen
life technologies

AccuPrime™ *Pfx* DNA Polymerase

Cat. No. 12344-024 **Size: 200 Reactions**
12344-032 **1000 Reactions**

Conc. 2.5 U/µl **Store at -20°C**

Description

AccuPrime™ *Pfx* DNA Polymerase is a proprietary enzyme preparation containing recombinant DNA polymerase from *Thermococcus* species strain KOD (1,2). This polymerase possesses a proofreading 3′ to 5′ exonuclease activity that provides higher fidelity than *Pfu* DNA polymerase (3). AccuPrime™ *Pfx* DNA Polymerase is a highly processive enzyme and possesses a fast chain extension capability. It is provided in an antibody-bound form that is inactive at ambient temperatures. The enzyme regains activity after the initial denaturation step at 94°C in PCR cycling, providing an automatic "hot start" that increases specificity, sensitivity, and yield, while allowing room temperature assembly (4).

10X AccuPrime™ *Pfx* Reaction Mix contains thermostable AccuPrime™ proteins, $MgSO_4$, and dNTPs. Thermostable AccuPrime™ proteins enhance specific primer-template hybridization during every cycle of PCR (5). The high specificity, fidelity, and yield offered by AccuPrime™ *Pfx* DNA Polymerase make it ideal for demanding PCR applications such as site-directed mutagenesis and PCR expression cloning.

Reagents are provided for 200 or 1000 amplification reactions of 50 µl each.

Component	200-Rxn kit	1000-Rxn kit
AccuPrime™ *Pfx* DNA Polymerase (2.5 U/µl)	100 µl	500 µl
50-mM Magnesium Sulfate	1 ml	2 × 1 ml
10X AccuPrime™ *Pfx* Reaction Mix	1 ml	5 × 1 ml

Unit Definition

One unit of AccuPrime™ *Pfx* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-insoluble material in 30 min at 74°C.

Part. no. 12344.pps Rev. date: 07/11/03

AccuPrime™ *Pfx* DNA Polymerase Storage Buffer
50-mM Tris-HCl (pH 8.0), 50-mM KCl, 1-mM DTT, 0.1-mM EDTA, stabilizers, and 50% (v/v) glycerol

Quality Control
AccuPrime™ *Pfx* DNA Polymerase is functionally tested in an amplification reaction using 100 ng of K562 genomic DNA. A DNA polymerization activity assay measures percent of DNA polymerase inhibition versus an uninhibited control. AccuPrime™ proteins are tested for absence of double- and single-strand endonuclease activity and absence of 5′ and 3′ exonuclease activity.

General Recommendations and Guidelines for PCR
PCR is a powerful technique capable of amplifying trace amounts of DNA. All appropriate precautions should be taken to avoid cross-contamination.

$MgSO_4$: $MgSO_4$ is included in the 10X AccuPrime™ *Pfx* Reaction Mix at a final concentration of 1 mM, which is sufficient for most templates. For further optimization, add 0.1 µl to 1.0 µl of 50-mM $MgSO_4$ (included in the kit) to the reaction.

dNTPs: dNTPs are included in the 10X AccuPrime™ *Pfx* Reaction Mix at a final concentration of 0.3 mM.

Annealing Temperature: The optimal annealing temperature should be 5–10°C lower than the T_m of the primers used; if necessary, gradually increase the annealing temperature by 2–3°C for higher specificity.

KCl: For difficult primer sets, prepare titrations of KCl (not included) at final concentrations of 20–50 mM for further optimization.

PCR Protocol

The following general procedure is suggested as a starting point when using AccuPrime™ *Pfx* DNA Polymerase in any PCR amplification.

1. Add the following components to an autoclaved microcentrifuge tube at either room temperature or on ice:

Component	Volume	Final Conc.
10X AccuPrime™ *Pfx* Reaction mix*	5 µl	1X
Primer mix (10 µM each)*	1.5 µl	0.3 µM each
Template DNA (10 pg–200 ng)	≥1 µl	As required
AccuPrime™ *Pfx* DNA Polymerase**	0.4–1 µl	1.0–2.5 units
Autoclaved, distilled water	to 50 µl	

*AccuPrime™ *Pfx* DNA Polymerase will not function in reactions that contain dUTP either in the primers or in the dNTP mix.

**For most targets, 1 unit is optimal. Higher concentrations may be inhibitory. More enzyme may be required for longer targets (>3 kb).

2. Mix contents of the tubes and overlay with mineral or silicone oil, if necessary. (Note: The oil overlay is unnecessary in thermal cyclers equipped with a heated lid.)
3. Cap the tubes and centrifuge briefly to collect the contents.
4. Denature the template for 2 min at 95°C. Perform 25–35 cycles of PCR amplification as follows:

Three-step cycling	Two-step cycling
Denature: 95°C for 15 s	Denature: 95°C for 15 s
Anneal: 55–64°C for 30 s	Extend: 68°C for 1 min per kb
Extend: 68°C for 1 min per kb	

Note: Two-step cycling can be used for long primers with high T_m.

5. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use.
6. Analyze the products by agarose gel electrophoresis and visualize by ethidium bromide staining.

References

1. Takagi, M., Nishioka, M., Kakihara, H., Kitabayashi, M., Inoue, H., Kawakami, B., Oka, M., and Imanaka, T. (1997) *Appli. Environ. Microbiol., 63,* 4504-4510.
2. Nishioka M, Mizuguchi H, Fujiwara S, Komatsubara S, Kitabayashi M, Uemura H, Takagi M, Imanaka T. (2001) *J. Biotechnol., 88,* 141–9.
3. Cline, J., Braman., and Hogrefe, H. H. (1996) *Nucleic Acid Res., 24,* 3546.
4. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology, 12,* 506.
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Exhibit D

Results : KOD (1 - 2 /2)

Other keywords

Koditschek

[pdf] p12.pdf

We are currently developing instruments by this technology and technologies combining DNA polymerase KOD-Plus*, our product of high speed and high degree of accuracy. Upon the completion, we will be able to shorten PCR from one or two hours to about ve minutes at the shortest. In recent years, there are pathogenic microbes such as resistant

http://www.toyobo.co.jp/e/annai/zaimu/annual/2005/p12.pdf

Enzymes and Kits for genetic engineering uses

(rTaq DNA Polymerase [Standard], rTth DNA Polymerase [Low cost & High RT activity], KOD DNA Polymerase [Fast & High fidelity], KOD Dash[Fast & Reliable], KOD -Plus- [Fast,High Fidelity & Hot Start]) ☆Kits for PCR,RT-PCR Amplifying PRO: takes rTaq DNA Polymerase, thermostable enzyme for PCR. RT-PCR high: Convenient kit for RT-PCR with M-MLV RTase

http://www.toyobo.co.jp/e/seihin/xr/biolineups/genetic.html

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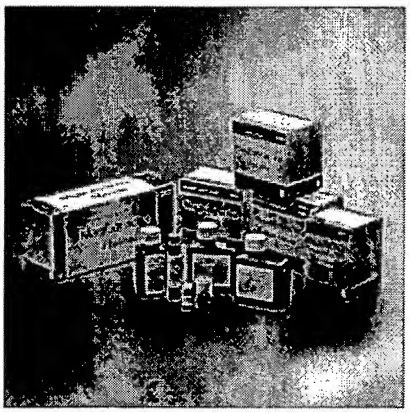
3. Enzymes and Kits for genetic engineering uses

In order to meet the requirements in the age of new-biotechnology,TOYOBO has developed a series of restriction enzymes, and modifying enzymes for genetic engineering, PCR, RT-PCR, kits for purification of nucleic acids and Electrophoresis equipment to make a major contribution in the field.

™Thermostable enzymes for PCR
Convenient and highly efficient modifying enzymes for PCR.
(rTaq DNA Polymerase [Standard], rTth DNA Polymerase [Low cost & High RT activity], KOD DNA Polymerase [Fast & High fidelity], KOD Dash®[Fast & Reliable], KOD -Plus- [Fast,High Fidelity & Hot Start])

™Kits for PCR,RT-PCR
Amplifying PRO:
takes rTaq DNA Polymerase, thermostable enzyme for PCR.
RT-PCR high:
Convenient kit for RT-PCR with M-MLV RTase and rTaq DNA Polymerase.
RT-PCR high -Plus-:
requires only one step for RT-PCR using rTth DNA Polymerase(for reverse transcription and PCR).

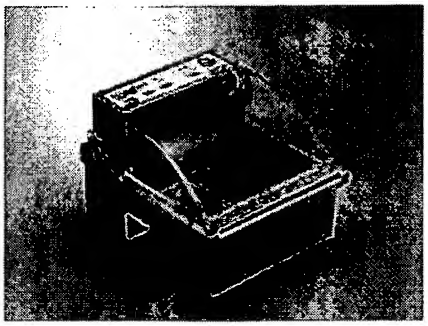
PCR Kits



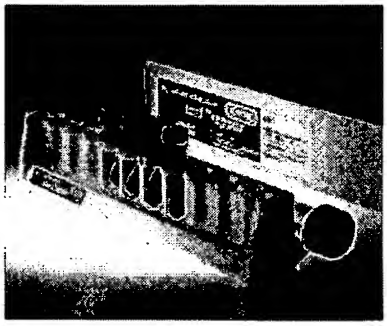
MagExtractor Series

™MagExtractor :Kits for purification of nucleic acids and protein
6 types of nucleic acid purification kits and 1 protein purification kit, with magnetic beads to bind nucleic acids or protein.
(Genome, total RNA, Plasmid, Plant Genome, Viral RNA, PCR product and DNA from agarose gel, and His-tagged protein. MagicalTrapper, a magnetic particle separator to be used to obtain high quality nucleic acids or tagged proteins.)

™GelMate®:
Submarine Agarose Gel Electrophoresis System with a timer and a fan to keep blur off on a display; A compact and convenient device for personal use in your lab.



GelMate®



Magical Trapper

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